

Tar intein incubation protocol

Goal: To check the intein splicing

Protocol:

3 ml O/N starter in TB 30C to:

*Tar1 (+CM)

*UU

*Tar1 intein2 (+CM)

1. Dilute 1:50 with TB and growth in 30°C to O.D(600nm) ~0.6* (Erlenmeyer 250 ml) . **Total volume:** 35 ml. (700 ul Bactria+ 34.3 ml TB)
2. Dilution with the bacteria: 0, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵. (to falcon 50ml)
0-w/o
0-Etoh
(7 tub per bacteria, 21 total)

| | Cons. 10 ⁻¹ | Cons. 10 ⁻² | Cons. 10 ⁻³ | Cons. 10 ⁻⁴ | Cons. 10 ⁻⁵ |
|-----------------------------|--|------------------------|------------------------|------------------------|------------------------|
| Bactria | 4.95 ml | 4.5 ml | 4.5 ml | 4.5 ml | 4.5 ml |
| Bactria with β-estradiol | 50 μl* β-estradiol fresh (C=10 mg/ml) | 500 μl | 500 μl | 500μl | 500μl |

| | | |
|------------|------------|--------------------|
| controls | with water | with absolute EtoH |
| bactria | 4.95 ml | 4.95 ml |
| water/EtoH | 50 μl | 50 μl |

3. Move to 32C for 2 hr (for intein splicing).

-NOTE: Meanwhile make plats according to "swarming assay" protocol.

4. After 2 hr do "swarming assay".

*according to the article (the assay: "Chemical detection with strain DIER and the YES system").